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TLC separation of lipids from larval instar gut contents of beetle pest, *Callosobruchus chinensis* L. propagating on gram and moong hosts

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Abstract : TBy the technique of TLC separation of lipids present in the gut content of the larval instars of Gram and Moong hosts (both uninfected and infected) were analysed. The TLC plates were subjected to sets of biomolecule specific separation solvent with their specific location differentiation reagents, the isolated spots gave different type of colour which were located.

Keywords : Gram , Moong, Larval instar ,TLC, C.chinensis Linn., BIomolecules.

INTRODUCTION

The biochemical composition of gut content of larval instar of *C.chinensis* L. Propagates on legumes like Gram and Moong host which shows the reflection of host specificity in terms of important nutritional metabolites. The investigation is an attempt to analyse the lipids present in the gut of larval instar of *C.chinensis* by TLC standard technique.[1,3,4,5]

The TLC analysis as done to isolate the variable numbers of lipids present in the gut content of the larval instar (1^{st} to 4^{th}) of *C.chinensis* L. Infesting on stored grains which displayed a significant qualitative and quantitative variation in the growing larvae of *C.chinensis* L. The no. of isolated spots in the gut content shows instar specific (1^{st} to 4^{th}) variation due to the enzymatic constitution of the developing larval gut which is responsible for breakdown and synthesis of the biomolecules.[7,9,11,12.]

METHODS AND MATERIALS

The thin layer chromatographic analysis of the extracted samples of both host (healthy and infected) the gut content of feeding larvae were done for lipids. The pest samples were treated with the following extraction solvents which acted elutants for the important biomolecules under investigation. The use of VLD – micro syringe was made in order to draw out the gut composition from both anterior and posterior end of the larval instars.

1. Elutants or Extraction solvent (ES)

Solvent were used for extraction of lipids. The samples were masseurated in borosil make mortar and pestle in the said solvent, least for at least twelve hours, homogenized and centrifuged. The supernatant liquid after sedimentation was used as the test sample to be charged on the activated silica gel plates .

2. Separation Solvent System (SSS)

For lipids: For separation of lipids constituents , following solvent ,media were tried one by one with a view to standardizing a particular solvent for better result.

Solvent no.1. Petroleum ether/diethyl : (90:10:1v/v)

Solvent no.2.Acetone (90:10:1v/v)

Solvent no.3.Isopropyl ether/acetic acid (24:1v/v)

The solvent Isopropyle ether/acetic acid (24:1v/v) proved to be better result yielding.

3.Spraying or Location Reagents

For lipids: In order to locate the spots of separated by the solvent, a specific detection spray of ethanolic 0.2% 2,7 dichlorofluorescein (developer) or 0.5% ethanolic rhodamine B or 6G was used and the plate was kept in

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oven at 100^0C.However,0.5% ethaqnolic rhodamine B proved to be better location reagent.

4.Sprayer and chromatographic jars

As most of the spraying or location reagents, solvents and elutants used were of nonpolar nature, so the specific glass sprayer and glass jar were used. Glass sprayer was made with the exhaust and delivery tube having manual pumping device.

5.Location of colours of the spots and measurement of Rf value.

The dried TLC plates subjected to sets of biomolecule specific separation solvent with their specific location / differentiation reagents, the isolated spots gave different types of colour which were easily located. More authentic protocol in chromatography has been given in terms of measurement of Rf (Resolution front) value through following formula:

Rf=Distance (mm/cm)travelled by an isolated spot from its origin

Max.Distance(mm/cm)travelled by the solvent from its origin

Rf value may differ according to variation in room temperature, thickness of absorbent layer and quantity of solute and standards.

OBSERVATION

In order to investigate the lipid present in the two host varieties Gram and Moong as well as those present in the larval gut contents for establishing the biochemical basis.

The lipid separated on the plates from the uninfected Gram and Moong host powder, in trial I, two spots from Gram host was isolated namely Phospholipid (Rf value 0.55,violet spot) and Triacylglyceride (Rf value 0.23,blue spot) and from Moong host 3 isolated spots were of Glycolipid (Rf value 0.49,Yellow spot), Phospholipid (Rf value 0.33,violet spot) and Triacyglyceride (Rf value 0.23,blue spot). In Trial II, three spots in Gram namely Glycolipid (Rf value 0.51,Yellow spot), Phospholipid (Rf value 0.33,Violet spot), Triacylglyceride(Rf value 0.23,blue spot) and in Moong Glycolipid I(Rf value 0.49,yellow spot), Glycolipid II(Rf value 0.42,yellow spot), Phospholipid (Rf value 0.33,violet spot), Triaclyglyceride (Rf value 0.23,blue spot) were located .(**Table 1A,Table 1B & Graph I, Graph II**)

In larval instar 1st gut content from Gram host,two spots namely Phospholipid (Rf value 0.33,violet spot), Triacylglyceride (Rf value 0.23,blue spot) and in 2nd larval instar,2 spots namely Glycolipid (Rf value 0.49,yellow spot) and Phospholipid (Rf value 0.33,violet spot)were isolated. Similarly from 3rd larval instar, only 2 spots of Glycolipid (Rf value 0.512,yellow spot), Phospholipid (Rf value 0.33,violet spot)were isolated Likewise 4th larval instar,4 spots Glycolipid-I (Rf value 0.57,yellow spot),Glycolipid –II(Rf value 0.49,yellow spot),Phospholipid (Rf value 0.33,violet spot)& Triacylglyceride (Rf value 0.23 ,blue spot) were obtained respectively.**(Table 2A,Table 2B &Graph III)**

The TLC analysis for lipids from the instar wise gut content larvae propagating on Moong host were namely, In 1st instar 3 spots namely Glycolipid (Rf value 0.51,yellow spot), Phospholipid (Rf value 0.33,violet spot) and Triaclgiyceride (Rf value 0.23,blue spot),in 2nd again 3 spots were isolated Glycolipid (Rf value 0.49,yellow spot), Phospholipid-I (Rf value 0.42,violet spot) and Phospholipid-II(Rf value 0.33,violet spot) in 3rd ,4 spots Glycolipid (Rf value 0.51,yellow spot), Phospholipid-I (Rf value 0.42,violet spot), Phospholipid-II (Rf value 0.33,violet spot) and Triacylglyceride (Rf value 0.23,blue spot) in the 4th ,3 spots of Glycolipid-I (Rf value 0.58,yellow spots) Glycolipid-II (Rf value 0.49,yellow spot), Phospholipid (Rf value 0.42,violet spot).(**Table 3A,Table 3B & Graph IV).**

Table 1 A : One dimension vertical-TLC Separation of lipids of uninfected Gram and Moong Host (Control) sponsoring the propagation of beetle *C.chinensis* L.

TRIAL 1

Spot Nos. in ascending order	GRAM			MOONG		
	Colour	Rf Value	Type	Colour	Rf Value	Type
1.	Violet	0.33	Phospholipid*	Yellow	0.49	Glycolipid
2.	Blue	0.33	Triacylglyceride*	Violet	0.33	Phospholipid*
3	-	-	-	Blue	0.23	Triacylglyceride*

Separation solvent : Isopropyle ether/ acetic acid(24:1v/v)

Location reagent : (a) 0.5% ethanolic rhodamine B

Table 1B : One dimension vertical – TLC Separation of lipids of unifected Gram and Moong Host (control) sponsoring the propagation of beetle *C.chinensis* L.

TRIAL 2

Spots Nos. In ascending order	GRAM			MOONG		
	Colour	Rf Value	Type	Colour	Rf Value	Type
1.	Yellow	0.51	Glycolipid	Yellow	0.49	Glycolipid
2.	Violet	0.33	Phospholipid*	Yellow	0.42	Glycolipid
3.	Blue	0.23	Triacylglyceride*	Violet	0.33	Phospholipid*
4.	-	-	-	Blue	0.23	Triacylglyceride*

Separation solvent : Isopropyle ether/ acetic acid (24:1 v/v)

Location reagent: (a) 0.5% ethanolic rhodamine B

Table 2 A: One dimension vertical –TLC Separation of lipids of gut content of larval instars of Gram beetle *C.chinensis* L. growing on Gram Host.

Spot Nos. in ascending order	INSTAR-I			INSTAR-II		
	Colour	Rf Value	Type	Colour	Rf Value	Type
1.	Violet	0.33	Phospholipid*	Yellow	0.49	Glycolipid
2.	Blue	0.23	Triacylglyceride*	Violet	0.33	Phospholipid*

Separation solvent : Isopropyle ether/acetic acid (24:1v/v)

Location reagent : (a) 0.5% ethanolic rhodamine B

Table 2 B: One dimension vertical – TLC Separation of lipids of gut content of larval instars of Gram beetle *C.chinensis* L. Growing on Gram Host.

Spot Nos. In ascending Order	INSTAR-III			INSTAR-IV		
	Colour	Rf Value	Type	Colour	Rf Value	Type
1.	Yellow	0.51	Glycolipid	Yellow	0.57	Glycolipid
2.	Violet	0.33	Phospholipid	Yellow	0.49	Glycolipid
3.	-	-	-	Violet	0.33	Phospholipid*
4.	-	-	-	Blue	0.23	Triacylglyceride*

Separation solvent : Isopropyle ether/acetic acid (24:1 v/v)

Location reagent : (a) 0.5% ethanolic rhodamine B

Table 3 A: One dimension vertical-TLC Separation of lipids of gut contents of larval instars of Gram beetle of *C.chinensis* L. Growing on Moong Host.

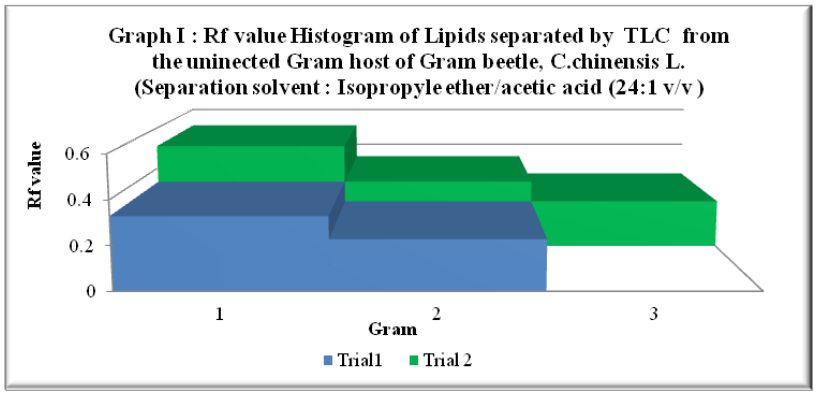
Spot Nos. In ascending order	INSTAR-I			INSTAR-II		
	Colour	Rf Value	Type	Colour	Rf Value	Type
1.	Yellow	0.51	Glycolipid	Yellow	0.49	Glycolipid
2.	Violet	0.33	Phospholipid*	Violet	0.42	Phospholipid*
3.	Blue	0.23	Triacylglyceride*	Violet	0.33	Phospholipid*

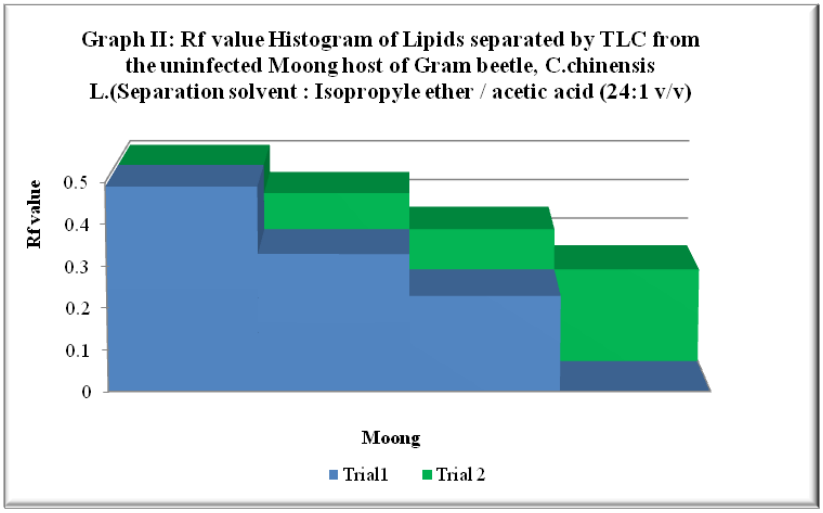
Separation solvent: Isopropyle ether/acetic acid (24:1 v/v)
Location reagent: (a) 0.5% ethanolic rhodamine B

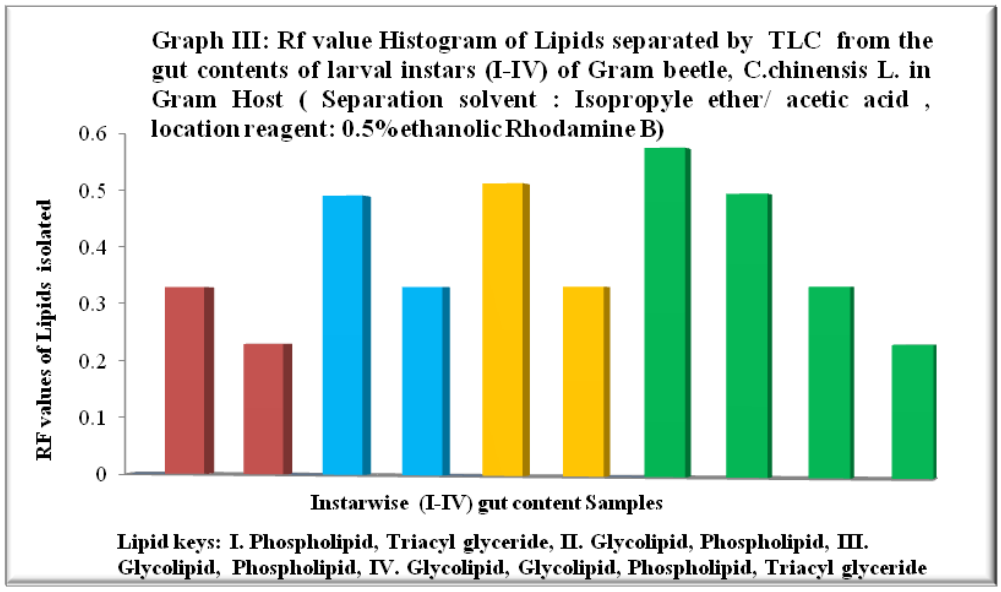
Table 3 B: One dimension vertical – TLC Separation of lipids of gut contents of larval instars of Gram beetle *C.chinensis* L. Growing on Moong Host.

Spot Nos. In ascending order	INSTAR-III			INSTAR-IV		
	Colour	Rf Value	Type	Colour	Rf Value	Type
1.	Yellow	0.51	Glycolipid	Yellow	0.58	Glycolipid
2.	Violet	0.42	Phospholipid*	Yellow	0.49	Glycolipid
3.	Violet	0.33	Phospholipid*	Violet	0.42	Phospholipid*
4.	Blue	0.23	Triacylglyceride*	-	-	-

Separation solvent : Isopropyle ether/ acetic acid (24:1 v/v)
Location reagent (a) 0.5% ethanolic rhodamine

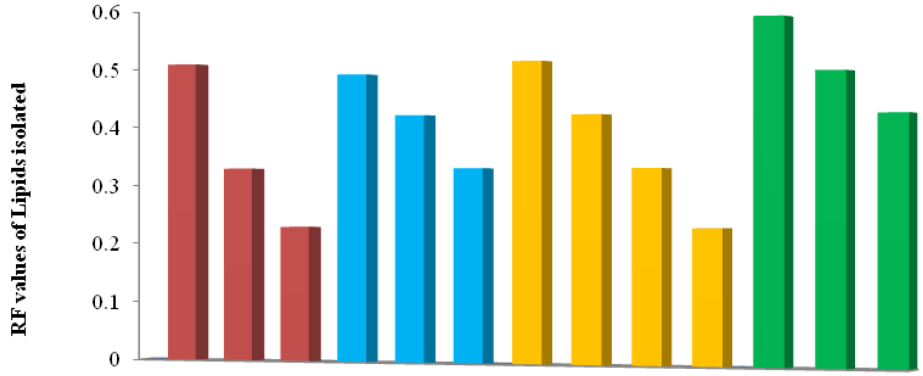






Graph III: Rf value Histogram of Lipids separated by TLC from the gut contents of larval instars (I-IV) of Gram beetle, C.chinensis L. in Gram Host (Separation solvent : Isopropyle ether/ acetic acid , location reagent: 0.5% ethanolic Rhodamine B)

Lipid keys: I. Phospholipid, Triacyl glyceride, II. Glycolipid, Phospholipid, III. Glycolipid, Phospholipid, IV. Glycolipid, Glycolipid, Phospholipid, Triacyl glyceride



Graph IV : Rf value Histogram of Lipids separated by TLC from the gut contents of larval instars (I-IV) of Gram beetle, C.chinensis L. in Moong Host (Separation solvent : Isopropyle ether / acetic acid and location reagent- 0.5% Ethanolic Rhodamine B

Lipid keys: I. Glycolipid, Phospholipid, Triacyl glyceride, II. Glycolipid, Phospholipid, Phospholipid III. Glycolipid, Phospholipid,Phospholipid, Triacyl glyceride IV. lycolipid, Glycolipid, Phospholipid,.

DISCUSSION

The analysis of lipids in the gut content of the feeding larval instars on the two host varieties Gram and Moong gives important information towards the specialization of the trophic relation of the pest attacking legumes.[2,6]

The specific findings of TLC separation of lipids of uninfected Gram host seed powder content provides a picture a available biomolecular of the host on which the pest has preferably thrive and propagated. As many as two types of lipids were isolated through TLC namely Phospholipid, Triacylglyceride with their respective Rf value and colours. As regard the biomolecular composition of the second host Moong seed powder in the uninfected condition in the TLC separation,3 varieties were isolated that were Glycolipid, Phospholipid and Triacyglyceride.

On examination of the gut content with TLC separation technique for lipids of 1st to 4th larval instar feeding and propagating on the Gram and Moong host ,an extensive picture of lipids were isolated. However the no. Of lipids isolated from the gut contents displayed a rising trend from 1st to the 4th instar larvae successively from the 2(1st instar),2(2nd instar),2(3rd instar),4(4th instar). Similarly the no. Of the lipids isolated also reflected a rising trend from 3 (1st instar),3(2nd instar),4(3rd instar)

and 3(4th instar). These bio molecules of particular nature either in the lipids as well as allele chemical substances play an important role in the establishment of the host specificity.[8,10]

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